

In the Claims:

1. (Previously Presented) A sample preparation method for a medium suspected of containing contaminants, the method comprising a) passing a known volume of said medium through a filter from an influent side to an effluent side in a filter device thereby concentrating the contaminants on the influent side of the filter in the filter device, b) contacting the influent side of the filter in the filter device with a liquid vehicle containing at least one substrate that through interaction with the contaminants each produces a detectable moiety, c) and allowing the substrate to interact with the contaminants on the influent side of the filter in the filter device for a period of time, which is sufficient to allow the detectable moiety to be detected in the liquid vehicle.
2. (Previously Presented) The method according to claim 1, wherein, prior to step a, the medium is passed through a prefilter that does not retain the contaminants, but retains larger particles.
3. (Currently Amended)The method according to claim ~~1 or 2~~, the contaminants are selected from the group consisting of bacteria; fungi, such as filamentous fungi and yeast; algae; protozoans; spores from bacteria; fungal spores; and pollen, and fragments thereof.
4. (Currently Amended)The method according to ~~any one of the preceding claims~~ 1, wherein the medium is a liquid medium.
5. (Previously Preseneted) The method according to claim 4, wherein the liquid medium is selected from the group consisting of environmental water, drinking water, hot water, industrial water, process water, cleaning in place water, a liquid extract of a solid material, a suspended or solubilised surface sample, and liquid industrial products such as cosmetics, pharmaceuticals, and foodstuffs.
6. (Currently Amended)The method according to claim ~~4-5~~, wherein the viscosity of the liquid medium is reduced prior to step a.

7. (Previously Preseneted) The method according to claim 6, wherein viscosity is reduced by means of dilution or by means of treatment with a chemical agent such as a solubility enhancing agent or a detergent.

8. (Currently Amended)The method according to ~~any one of~~ claims 1-3, wherein the medium is a gaseous medium.

9. (Previously Preseneted) The method according to claim 8, wherein the gaseous medium is air, such as air from a sterile facility, a laminar air-flow device or environmental air.

10. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the filter has a pore size small enough so as to retain substantially all contaminants in the medium.

11. (Previously Preseneted) The method according to claim 10, wherein the filter has a pore size large enough to let the detectable moiety pass through the filter.

12. (Previously Preseneted) The method according to claim 11, wherein the pore size is at most 20 μm .

13. (Currently Amended)The method according to claim 11 ~~or 12~~, wherein the pore size is at least 0.1 μm .

14. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the at least one substrate produces the detectable moiety by being cleaved by an enzyme that is characteristic for the contaminants.

15. (Previously Presented) The method according to claim 14, wherein the enzyme is selected from the group consisting of carbohydrases, proteases, lipases, esterases, amidases, sulfatases, nucleases and phosphatases such as alkaline phosphatase.

16. (Currently Amended) The method according to claim 14 ~~or 15~~, wherein the enzyme is expressed constitutively by microorganisms.

17. (Currently Amended) The method according to ~~any one of claims 14-16~~ wherein the at least one substrate is a fluorogenic or chromogenic substrate producing blue, green and red fluorescent products as the detectable moiety.

18. (Currently Amended) The method according to ~~any one of claims 14-17~~, wherein the at least one substrate is selected from the group consisting of 5-bromo-4-chloro-3-indolyl phosphate disodium salt; 9h-(1,3-dichloro-9,9-dimethylacridine-2-one-7-yl) phosphate ammonium salt; fluorescein diphosphate tetraammonium salt; a methyl umbelliferyl derivative such as 6,8-difluoro-4-methylumbelliferyl phosphate, 4-methylumbelliferyl phosphate dicyclohexylammonium salt trihydrate, 4-methylumbelliferyl phosphate free acid; 4-methylumbelliferyl phosphate dilithium salt, 4-methylumbelliferyl- β -N-acetylglucosaminide, and trifluoromethylumbelliferyl phosphate; salts of 4-nitrophenyl phosphate; and resorufin phosphate.

19. (Currently Amended) The method according to ~~any one of claims 14-18~~, wherein the detectable moiety is detectable in an amount of at the most 100 picomoles, preferably at the most 50 picomoles, more preferably at the most 20 picomoles and even more preferably at the most 10 picomoles and most preferably at the most 1 picomoles.

20. (Currently Amended) The method according to claim 1 ~~any one of the preceding claims~~, wherein at least two substrates are used that produce detectable moieties providing signals that can be combined into one single measured signal value.

21. (Currently Amended)The method according to ~~any one of~~ claims 1-20, wherein at least two substrates are used that produce detectable moieties providing distinguishable signals.

22. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the contaminants are viable microorganisms.

23. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the amount of substrate in the liquid vehicle does not limit the rate of production of the detectable moiety.

24. (Previously Presented) The method according to claim 23, wherein the rate of production of the detectable moiety is a function of the quantity of contaminants in the known volume of the medium.

25. (Previously Presented) The method according to claim 24, wherein the function is linear.

26. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein several different known volumes of the medium are each passed through a filter in step a, so as to ensure that at least one of the volumes contains a suitable number of contaminants.

27. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the filter is part of a closed, sterile filter device.

28. (Previously Presented) The method according to claim 27, wherein the closed, sterile filter device is disposable.

29. (Currently Amended)The method according to claim 27-~~or 28~~, wherein the closed, sterile filter

device integrates the filter and a filter housing into one irreversibly closed structural unit.

30. (Currently Amended)The method according to ~~any one of claims 27-29~~, wherein the longest cross-sectional axis of the closed, sterile filter device does not exceed a length of 10 cm.

31. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, wherein the interaction in step c is terminated by interrupting the contact between the substrate and the contaminants.

32. (Previously Presented) The method according to claim 31, wherein interruption is obtained by evacuating the liquid vehicle from the filter device while retaining the contaminants in the filter device,

33. (Previously Presented) The method according to claim 32, wherein the liquid vehicle is evacuated from the filter device in the direction from the influent to the effluent side of the filter.

34. (Previously Presented) The method according to claim 33, wherein evacuation is obtained by applying an elevated pressure on the influent side of the filter or by applying a lowered pressure on the effluent side of the filter.

35. (Currently Amended)The method according to ~~any one of claims 1-30~~, wherein the interaction in step c is terminated on the filter or wherein the interaction is not terminated.

36. (Currently Amended)The method according to claim 1~~any one of the preceding claims~~, comprising, after step c, a further step d) that entails detecting, quantitatively or qualitatively, the detectable moiety in the liquid vehicle and correlating the detection of the moiety to the amount or presence of contaminants in the sample.

37. (Previously Presented) The method according to claim 36, wherein detection in step d is performed by measuring fluorescence characteristic of the detectable moiety.

38. (Previously Presented) The method according to claim 37, wherein the fluorescence in step d is measured directly on the liquid vehicle without an interruption of the contact between the liquid vehicle and the contaminants.

39. (Currently Amended) The method according to ~~any one of~~ claims 36-38, wherein the correlation in step d comprises the use of a pre-determined standard curve that expresses the relationship between the amount of contaminants and the amount of the detectable moiety under standard conditions.

40. (Currently Amended) The method according to ~~any one of~~ claims 36-39 wherein detection is performed in a microtiter system.

41. (Currently Amended) The method according to claim 1 ~~any one of the preceding claims~~, wherein the contaminants are subjected to a signal-enhancing influence, either prior to step a or in step b.

42. (Previously Presented) The method according to claim 41, where the signal-enhancing influence increases the overall sensitivity in a subsequent detection or favours subsequent detection of specific types of contaminants, or reduces detection of specific types of contaminants.

43. (Previously Presented) The method according to claim 41, wherein the signal-enhancing influence is selected from an enzyme-enhancing substance, a selective temperature or temperature range, a selective pH, a selective salt concentration, a non-selective growth-enhancer, and a selective growth-enhancing substance.

44. (Currently Amended) The method according to claim 1 ~~any one of the preceding claims~~, wherein step a is preceded by an incubation of the medium.

45. (Previously Presented) The method according to claim 44, wherein the incubation entails

- treatment with an enzyme inducing substance thereby enhancing the detection of the detectable moiety, and/or
- subjecting the medium to a selective substance for yeast, fungi or bacteria, and/or -
- subjecting the medium to a non-selective growth-enhancer for microorganisms, and/or
- subjecting the medium to a substance capable of extracting cellular enzymes.

46. (Previously Presented) A kit for determination of contaminants in a medium, the kit comprising

- at least one sterile filter device comprising a filter with a pore size sufficiently small to

retain the contaminants on the filter's influent side,

- means for passing a known volume of medium through the filter,
- at least one agent that upon interaction with the contaminants will release a detectable moiety, the amount of which can be correlated with the amount of contaminants that have interacted with the agent, and
- instructions that sets forth steps for a) obtaining a known volume of medium and passing it through the sterile filter device, b) contacting the influent side of the filter in the filter device with the agent, c) allowing the agent to interact with contaminants that might be on the influent side of the filter in the filter device, and d) quantitatively detecting the detectable moiety.

47. (Previously Presented) Use of a closed, sterile filter device as a reaction vessel for a reaction between contaminants retained in the device and a substrate that releases a detectable moiety when contacted with the contaminants.